Influence of blinded wound closure on the volume stability of different GBR materials: an in vitro cone-beam computed tomographic examination. Part I

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Abstract: OBJECTIVE To test whether the use of (i) particulated bone substitute + collagen membrane used for guided bone regeneration (GBR) of peri-implant bone defects renders different results from (ii) particulated bone substitute + collagen membrane + fixation pins and from (iii) block bone substitute + collagen membrane with respect to the volume stability of the augmented region during suturing of mucosal flaps. MATERIAL AND METHODS Twenty peri-implant box-shaped bone defects were created in 10 pig mandibles. Every bone defect was augmented once with each of the following GBR procedures: Granulate (particulated xenograft + collagen membrane), Granulate + Pins (particulated xenograft + collagen membrane + fixation pins), and Block (block xenograft + collagen membrane). Cone-beam computed tomography scans were obtained prior and after blinded wound closure. The horizontal thickness (HT) of the augmented region (bone substitute + membrane) was assessed at the implant shoulder (HT0 mm) and at 1-5 mm apical to the implant shoulder (HT1 mm -HT5 mm). The changes of HT during flap suturing were calculated as absolute (mm) and relative values (%). Repeated-measures ANOVA was used for statistical analysis. RESULTS Wound closure induced a statistically significant change of HT0 mm and of HT1 mm in all the treatment groups ($P \leq 0.05$). The change in HT0 mm measured -42.8 ± 17.9% (SD) for Granulate, -22.9 ± 21.2% (SD) for Granulate + Pins, and -20.2 ± 18.9% (SD) for Block. The reduction in HT0 mm , HT1 mm , HT2 mm , and HT3 mm for the Granulate procedure was significantly higher as compared to the Granulate + Pins and the Block procedures ($P \leq 0.05$). There were no statistically significant differences in the change of HT between the Granulate + Pins and the Block procedures ($P > 0.05$). CONCLUSION Wound closure induced displacement of the bone substitute resulting in a partial collapse of the collagen membrane in the coronal portion of the augmented site. The stability of the bone substitute and collagen membrane was enhanced by the application of fixation pins and by the use of block bone substitute instead of particulated bone substitute.

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Abstract

Objective: To test whether the use of (1) particulated bone substitute + collagen membrane used for guided bone regeneration (GBR) of peri-implant bone defects renders different results from (2) particulated bone substitute + collagen membrane + fixation pins and from (3) block bone substitute + collagen membrane with respect to the volume stability of the augmented region during suturing of mucosal flaps.

Material and methods: Twenty peri-implant box-shaped bone defects were created in 10 pig mandibles. Every bone defect was augmented once with each of the following GBR procedures: Granulate (particulated xenograft + collagen membrane), Granulate + Pins (particulated xenograft + collagen membrane + fixation pins) and Block (block xenograft + collagen membrane). Cone beam computed tomography scans were obtained prior and after blinded wound closure. The horizontal thickness (HT) of the augmented region (bone substitute + membrane) was assessed at the implant shoulder (HT_{0mm}) and at 1 mm to 5 mm apical to the implant shoulder (HT_{1mm} - HT_{5mm}). The changes of HT during flap suturing were calculated as absolute (mm) and relative values (%). Repeated measures ANOVA were used for statistical analysis.

Results: Wound closure induced a statistically significant change of HT_{0mm} and of HT_{1mm} in all the treatment groups ($p \leq 0.05$). The change in HT_{0mm} measured -42.8 ± 17.9% (SD) for Granulate, -22.9 ± 21.2% (SD) for Granulate + Pins and -20.2 ± 18.9% (SD) for Block. The reduction in HT_{0mm}, HT_{1mm}, HT_{2mm} and HT_{3mm} for the Granulate procedure was significantly higher as compared to the Granulate + Pins and the Block procedures ($p \leq 0.05$). There were no statistically significant differences in the change of HT between the Granulate + Pins and the Block procedures ($p > 0.05$)

Conclusion: Wound closure induced displacement of the bone substitute resulting in a partial collapse of the collagen membrane in the coronal portion of the augmented site. The stability of the bone substitute and collagen membrane was enhanced by the application of fixation pins and by the use of block bone substitute instead of particulated bone substitute.

Introduction

Edentulous jaw regions frequently present a reduced dimension of the alveolar ridge, either due to congenital or post-inflammatory defects or resulting from post-extraction ridge resorption (Tan et al. 2012). The prosthetically driven implant placement is, therefore, often associated with the presence of peri-implant bone dehiscences and fenestrations.

Guided bone regeneration (GBR) is the best-documented method used to augment bone in localized alveolar defects (Benic & Hämmerle 2014). A large number of preclinical and clinical trials demonstrated that exposed implant surfaces can successfully osseointegrate following GBR procedures (Kohal et al. 1999, Palmer et al. 1998, Warrer et al. 1991, Wilson et al. 1998, Becker et al. 1991). Moreover, there is a large body of clinical evidence documenting that survival rates of dental implants placed in conjuction with GBR are similar to survival rates of implants entirely placed into the native bone (Mayfield et al. 1998, Zitzmann et al. 2001, Benic et al. 2009, Zumstein et al. 2010, Jung et al. 2012).

The application of particulated deproteinized bovine-derived bone mineral (DBBM) covered with resorbable collagen membrane is, currently, the most widely used and best documented method for augmentation of dehiscence- and fenestration-type bone defects (Chiapasco et al. 2009, Jensen & Terheyden 2009). DBBM and native collagen membranes exhibit good tissue integration, rendering high clinical success and low complications rates of the GBR procedures (Benic & Hämmerle 2014). However, major drawback of particulated grafting materials and collagen membranes may be caused by their unfavourable mechanical properties with poor resistance to collapse. While suturing the mucosal flap or during the healing phase, compressive forces at the augmented site may result in membrane collapse and displacement of parts of the grafting material (Mellonig et al. 1998, Schwarz et al. 2007, Strietzel et al. 2006, Zellin et al. 1995).

Some publications pointed out that the volume stability of sites that are augmented by GBR may be affected by properties of the grafting material and of the membrane, by use of pins for the stabilization of membranes, by flap manipulation and type of temporary restoration (Von Arx et al. 2001, Carpio et al. 2000, Zitzmann & Marinello 1999, Lorenzoni et al. 1998). There is, however, limited evidence available on the short- and the long-term three-dimensional changes of jaw regions augmented by means of GBR (Benic & Hämmerle 2014).

The primary aim of the present study was to test whether the flap suturing following GBR of peri-implant bone defects by using xenografts and collagen membranes induces, as assessed by means of cone-beam computed tomography (CBCT), a displacement of the grafting material. In addition, it was tested whether the use of (1) particulated bone substitute + collagen membrane renders different results from (2) particulated bone substitute + collagen membrane + fixation pins and from (3) block bone substitute + collagen membrane with respect to the volume stability of the augmented region during suturing of mucosal flaps.

Material and methods

Two clinicians performed the experimental surgical interventions. The first operator created the peri-implant defects and performed GBR. The second clinician, that was unaware of the treatment strategy used for GBR and of the aim of the study, provided the wound closure, in order to eliminate operator's bias.

In vitro model

Ten mandibles were obtained from five month old pigs. Crestal incisions were bilaterally performed mesial to the second premolars and one vertical releasing incision was made at the disto-buccal aspect of each second premolar. Mucoperiosteal flaps were elevated, second premolars were hemi-sectioned and their mesial roots were extracted. Twenty box-shaped bone defects, one at each extraction site, were prepared by means of cylindrical carbide drills. The bone defects measured 8 mm mesio-distally, 3 mm bucco-orally and 6 mm apico-coronally (Fig. 1). One 8 mm-length and 4 mm-diameter titanium implant (OsseoSpeed™ S, ASTRA TECH Implant System, DENTSPLY Implants, Mannheim, Germany) was inserted into each bone defect by placing the implant central axis along the lingual bone wall at the same distance from the mesial and the distal walls of the defect. The apico-coronal position of the implant shoulder corresponded to the most coronal part of the lingual bone wall. The distance between the implant surface and the most buccal aspect of the apical bone wall, in a direction perpendicular to the axis of the implant, measured 1 mm (Fig. 1).

GBR and wound closure

Prior to GBR, bone substitute materials were soaked in 50% aqueous solution of a radio-opaque contrast medium (Gastrografin®, Bayer, Zurich, Switzerland).

Every bone defect (n = 20) was augmented once for each GBR procedure under investigation. The sequence of the application was randomly assigned by casting a die.

The following GBR procedures were tested (Fig. 2):

- ***Granulate***: Particulated Demineralized Bovine Bone Mineral (DBBM) (Bio-Oss® granules 0.25-1 mm, Geistlich Pharma AG, Wolhusen, Switzerland) + collagen membrane (Bio-Gide®, Geistlich Pharma AG) (n = 20)
- ***Granulate + Pins***: Particulated DBBM (Bio-Oss® granules 0.25-1 mm, Geistlich Pharma AG) + collagen membrane (Bio-Gide®, Geistlich Pharma AG) + two titanium fixation pins (Frios®, DENTSPLY Implants) (n = 20)
- ***Block***: Block DBBM (Bio-Oss® block, Geistlich Pharma AG) + collagen membrane (Bio-Gide®, Geistlich Pharma AG) (n = 20)

Bone substitutes were applied aiming to achieve 1 mm of over-contour with respect to the buccal surface of the alveolar ridge. A customized silicone guide was used to enable the application of a standardized amount of grafting material. For *Block* procedure, a block DBBM was individually shaped and adapted to fit the bone defect by using cylindrical carbide drills. The collagen membrane was applied to cover the bone substitute and overlap the walls of the defect by at least 2 mm. For *Granulate + Pins* procedure, two titanium pins were placed 1 mm apically to the apical wall of the defect in order to stabilize the collagen membrane (Fig. 2).

A periosteal release incision was performed in the apical portion of the buccal mucoperiosteal flap. The flaps were sutured with a polyamid monofilament suture (Dafilon® 5-0, B. Braun Medical AG, Sempach, Switzerland). One operator, that was unaware of the treatment strategy used for GBR, performed the suturing procedure in a standardized way (one horizontal mattress and four single interrupted sutures per site) (Fig 2).

Prior to the subsequent GBR procedure, the sutures, the membrane, the pins and the bone substitute were removed and the experimental site was rinsed with a 0.9% saline solution.

CBCT scanning

CBCT scans (I-Cat®, KaVo Dental GmbH, Biberach, Germany) of the mandible were performed immediately prior and after the flap suturing at each site. For the scanning procedure, the jaws were positioned on the supporting plate provided by the manufacturer with the occlusal plane parallel to the horizontal plane and positioned in the centre of field of view (FOV) using the laser orientation beams. The CBCT scans were obtained with the following technical parameters: 120 kV acceleration voltage, 5 mA beam current, FOV diameter of 16 cm, FOV height of 6

cm, 600 projections, 360º rotation, voxel size of 0.25 mm and scan time of 14.7 seconds (Benic et al. 2013).

CBCT image evaluation

OsiriX™ imaging software (OsiriX v.4.0 32-bit, Pixmeo SARL, Bernex, Switzerland) was used for the evaluation of the CBCT DICOM datasets. "Full dynamic" visualization modality was used to set the window level (3084) and window width (8168). Cross-sectional images perpendicular to the implant central axis and mandibular panoramic curve were used for the measurements. The horizontal thickness of the augmented region (bone substitute + membrane) was assessed in a direction perpendicular to the implant surface at the implant shoulder (HT_{0mm}) and at 1 mm, 2 mm, 3 mm, 4 mm and 5 mm apical to the implant shoulder (HT_{1mm} - HT_{5mm}) (Fig. 3). To facilitate the reproducibility of the measurements, a transparent acetate foil with printed implant outlines and levels for the assessment of HT was placed on the computer monitor over the CBCT images (Benic et al 2013).

The presence of void spaces within the augmented area was assessed to describe the fit of the bone substitute to the bone defect. Void spaces were defined as radio-lucent regions within the augmented area with a diameter ≥0.5 mm. One calibrated investigator performed all the CBCT measurements.

Data analysis

The changes of HT during flap suturing were calculated as absolute (mm) and relative values (%) (SPSS version 20, IBM, Armonk, USA).

Descriptive statistics were computed for all the parameters. For continuous parameters, the data distributions were represented with barplots and boxplots. The data were reported by using means, standard deviations (SD) and 95% confidence intervals (CI). The assumption of normality was controlled by Kolmogorov-Smirnov and Shapiro-Wilk tests. All the results, with exception of HT_{5mm} in the Block group, presented a normal distribution. For discrete variables, the absolute and the relative frequencies were calculated.

Repeated measures ANOVA were applied to detect differences of HT before suturing and differences of the changes in HT between the treatment procedures. The

Greenhouse-Geisser correction was performed when Mauchly's test ruled out sphericity. Results of tests with p-values ≤ 0.05 were considered statistically significant. In case of non-normal data distribution, non-parametric paired Wilcoxon test with Bonferroni correction of the significance level was applied to test differences between the treatment procedures. Results of tests with p-values ≤ 0.05/3 = 0.016 were considered statistically significant.

Two weeks after the CBCT image analysis, 10 randomly selected CBCT images were re-assessed to test the intra-observer reliability of CBCT measurements. The intra-class correlation coefficient (ICC) for HT_{0mm} - HT_{5mm} ranged from 0.973 to 0,989 (95% CI: 0,932 – 0,996), indicating high intra-examiner agreement.

Results

There were no statistically significant differences in HT before suturing between the treatment procedures ($p = 0.276$) (Table 1a). Suturing of mucosal flaps induced a statistically significant change in HT ($p = 0.001$) (Table 1b). The differences in the change of HT between the GBR procedures reached statistical significance ($p < 0.001$) (Table 1b).

The results of HT before and HT after suturing and the change in HT for different GBR procedures are presented in Tables 2 and 3 and Figures 4 and 5. The change in HT_{0mm} measured -42.8 ± 17.9% (SD) for Granulate, -22.9 ± 21.2% (SD) for Granulate + Pins group and -20.2 ± 18.9% (SD) for Block. The alteration of HT_{1mm} amounted to -23.4 ± 11.9% (SD) for Granulate, -6.9 ± 12.5% (SD) for Granulate + Pins and -10.8 ± 13.2% (SD) for Block. The reductions of HT_{0mm} and of HT_{1mm} were statistically significant in all the treatment groups (Granulate HT_{0mm}, $p < 0.001$; Granulate HT_{1mm}, $p < 0.001$; Granulate + Pins HT_{0mm}, $p < 0.001$; Granulate + Pins HT_{1mm}, $p = 0.012$; Block HT_{0mm}, $p < 0.001$, Block HT_{1mm}, $p = 0.002$). The reduction of HT_{2mm} reached statistical significance for Granulate and Block (Granulate HT_{2mm}, $p < 0.001$; Block HT_{2mm}, $p = 0.05$). The reduction of HT_{3mm} and of HT_{4mm} was statistically significant only for Granulate (Granulate HT_{3mm}, $p < 0.001$; Granulate HT_{4mm}, $p = 0.012$) (Table 2 and Fig. 4).

The differences in the changes of HT_{0mm}, HT_{1mm}, HT_{2mm} and HT_{3mm} between Granulate and Granulate + Pins (HT_{0mm}, $p < 0.001$; HT_{1mm}, $p < 0.001$; HT_{2mm}, $p = 0.001$; HT_{3mm}, $p = 0.007$) and between Granulate and Block (HT_{0mm}, $p = 0.011$; HT_{1mm}, $p = 0.004$; HT_{2mm}, $p = 0.008$; HT_{3mm}, $p = 0.013$) reached statistical significance. There were no statistically significant differences in the change of HT between Granulate + Pins and Block (HT_{0mm}, $p = 0.498$; HT_{1mm}, $p = 0.210$; HT_{2mm}, $p = 0.216$; HT_{3mm}, $p = 0.362$; HT_{4mm}, $p = 0.913$; HT_{5mm}, $p = 0.760$) (Table 3 and Fig. 5).

The results regarding presence of voids within the augmented region are presented in Table 4. The majority of the voids were detected in Block group at 3 mm and 4 mm apical to the implant shoulder (Table 4).

Discussion

In the present *in vitro* study, suturing of mucosal flaps after GBR of peri-implant bone defects induced a considerable displacement of particulated grafting material, resulting in a partial collapse of collagen membrane. The displacement of the grafting material and of the membrane was mostly pronounced in the coronal portion of the augmented site at the level of the implant shoulder. These results demonstrate that, even though a clinically tension-free flap closure was achieved in all cases, compressive forces on the coronal portion of the augmented site during suturing could not be totally avoided.

The use of fixation pins in combination with particulated bone substitute and collagen membrane and the application of block bone substitute with collagen membrane performed significantly better with regards to the dimensional stability of the augmented site, as compared to GBR by means of particulated bone substitute and collagen membrane. The additional use of fixation pins or the use of block instead of particulated grafting material permitted to reduce the amount of membrane collapse at the level of implant shoulder by more than 50% (from -1.1 mm to -0.5 mm). These results show that the stability of the augmented site can be enhanced either by stabilizing the barrier membrane or by providing adequate support to the membrane through a stable bone substitute.

In the present study, the use of pins for membrane fixation considerably facilitated the clinical handling during GBR. The adaptation of the membrane and the stabilization of the grafting material in the desired position were enhanced through the application of two fixation pins apical to the bone defect.

In a previous clinical study, GBR procedures with resorbable or non-resorbable membranes were performed with or without the use of polylactide pins (Carpio et al. 2000). When membrane fixation was provided, a significantly higher success of GBR was found in terms of frequency of postoperative complications and reduction in the size of the peri-implant defects, as compared to GBR without membrane fixation. There is, however, limited evidence available about the effect of the use of pins for membrane stabilization on the three-dimensional stability of jaw regions augmented by means of GBR (Benic & Hämmerle 2014). Currently, the routine use

of fixation pins is not generally recommended in combination with collagen membranes (Buser 2009).

In the present investigation, the use of block bone substitute for GBR was associated with an enhanced dimensional stability of the augmented site, as compared to GBR with particulated grafting material. The clinical handling of DBBM block was, however, frequently associated with intra-operative complications, such as block fracture during the preparation and the adaptation to the defect. In case of fracture, a new block was prepared. The frequent presence of voids within the augmented region in CBCT images can be explained by the difficulty in achieving an accurate fit of the block within the bone defect. DBBM blocks were not stabilized with fixation screws. The reduction of HT in the coronal region and the similar percentage of HT increase in the apical area can be explained by the rotation of the block within the defect. Moreover, this fact explains the occurrence of the outlier values of change in HT in the apical region of the defect and the increase in the frequency of voids for the Block procedure.

There are only limited clinical data reporting on the application of DBBM block in combination with collagen membrane for GBR. In a clinical study, DBBM blocks and collagen membranes were applied to 12 patients to treat horizontal bone defects before implant placement (Hämmerle et al. 2008). After 9–10 months, in 11 of 12 patients the resulting bone volume was sufficient to allow implant placement in the prosthetically optimal position. It was therefore concluded that the procedure was effective for horizontal ridge augmentation. These results are in agreement with a preclinical study comparing autogenous bone blocks with DBBM blocks for horizontal ridge augmentation, in which a similar increase of ridge augmentation was clinically measured in both groups (De Santis et al. 2012). In fact, 3 months after GBR all sites treated with DBBM blocks appeared, clinically, to be suitable for implant placement. Histologically, however, several studies found that DBBM blocks were mainly embedded in connective tissue and only a moderate amount of new bone formation was observed in peripheral parts of the graft (De Santis et al. 2012, Schwarz et al. 2008, Schwarz et al. 2010).

In the present trial, an attempt to mimic the clinical situation of implant placement with simultaneous GBR was done. An *in vitro* model based on box-shaped peri-implant bone defects in pig mandibles was used for this purpose. The self-contained component of the peri-implant defect measured 1 mm in the bucco-oral direction. A small over-augmentation of the dehiscence defect is generally recommended when

using particulated grafting material and non-stable membranes, to compensate for the displacement of parts of the grafting materials (Benic & Hämmerle 2014). Bone substitutes were, therefore, applied in an attempt to achieve 1 mm of over-contour with respect to the buccal surface of the alveolar ridge.

A limitation of the present study was its *in vitro* set-up, which only partially simulated a clinical situation of GBR at peri-implant defects. Blood clot formation could not be reproduced in such an in vitro set-up. However, even though coagulation plays a role in the early healing, it cannot prevent the displacement of the bone substitute during wound closure. On the other hand, the *in vitro* model allowed standardizing the design of the mucosal flap, the morphology of the bone defect and the clinical procedures (e.g. amount of bone substitute, suturing technique). It was, therefore, possible to reduce the influence of confounding factors on the result of the procedures under investigation. Moreover, every site was treated once with each one of the three GBR procedures under investigation. This allowed further reducing the amount of the confounding factors, in particular the flap tension. Finally, one clinician, which was blinded and unaware of the aim of the study, performed the standardized wound closure in order to eliminate the operator's bias.

The clinical decision making regarding the choice of the optimal bone augmentation protocol and the selection of the materials is primarily based on the defect morphology and on whether or not the ridge contour needs to be augmented (Benic & Hämmerle 2014). Based on the data of the present study, it can be deduced that a partial collapse of the barrier membrane can be expected for augmentations performed with particulated bone substitutes and collagen membrane. Therefore, when using particulated bone substitutes and non-stable membranes, an over-augmentation of the defect is recommended to compensate for material displacement. In situations requiring an enhanced stability of the augmented site, the use of pins for membrane stabilization is recommended. GBR by means of customized DBBM blocks is to be considered a promising approach to provide an adequate support of the membrane.

Further investigations are needed to examine the clinical implications of the findings of the present study. Future research should determine the need for augmentation procedures regarding the long-term success of the implants. In addition, the long-term stability of the augmented bone should be assessed and monitored.

Conclusions

Within the limitations of the present *in vitro* study, it can be concluded that for GBR of peri-implant bone defects:

- Manipulation of mucosal flaps during suturing induced a displacement of bone substitute, resulting in a partial collapse of collagen membrane in the coronal portion of the augmented site.
- The primary stability of particulated bone substitute covered with collagen membrane was enhanced by applying pins for the fixation of the membrane.
- Block bone substitute in combination with collagen membrane performed significantly better than particulated bone substitute covered with collagen membrane in terms of dimensional stability of the augmented site during flap suturing.

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Table legend

Table 1a. Results of two-factor repeated measures ANOVA for horizontal thickness of the augmented region before suturing (baseline)

Table 1b. Results of two- and three-factor repeated measures ANOVA for horizontal thickness of the augmented region

Table 2. Results of horizontal thickness of the augmented region (HT) and change in HT at different apico-coronal levels for (a) Granulate, (b) Granulate + Pins and (c) Block procedures

Table 3. Results of change in horizontal thickness of the augmented region for different treatment procedures together with the results of repeated measures ANOVA

Table 4. Frequency of appearance of voids ≥ 0.5 mm within the augmented region in CBCT images

Figure legend

Fig. 1. (a) Buccal and (b) occlusal view of the experimental peri-implant bone defect

Fig. 2. (a) Particulated xenograft applied for the Granulate and the Granulate + Pins treatment modalities. (b) Block xenograft used for the Block treatment modality. (c) Collagen membrane applied for the Granulate and the Block treatment modalities. (d) Collagen membrane stabilized by two titanium pins used for the Granulate + Pins treatment modality. (e) Buccal view after suturing.

Fig. 3. Bucco-oral CBCT reconstructions with the measurements of the dimensions of the augmented regions (HT_{0mm} – HT_{5mm}). (a) Granulate, (b) Granulate + Pins and (c) Block treatment procedures before suturing. (d) Granulate, (e) Granulate + Pins and (f) Block treatment procedures after suturing.

Fig. 4. Bar plots representing the horizontal thicknesses of the augmented regions at different apico-coronal levels (HT_{0mm} – HT_{5mm}) before and after suturing for (a) Granulate, (b) Granulate + Pins and (c) Block treatment procedures.

Fig. 5. Box plots representing the changes of horizontal thicknesses of the augmented regions during suturing for Granulate, Granulate + Pins and Block treatment procedures (a) in mm and (b) in %. ◦ and * in the figure represent the outliers. One outlier value of HT_{5mm} for the Block procedure is not represented.

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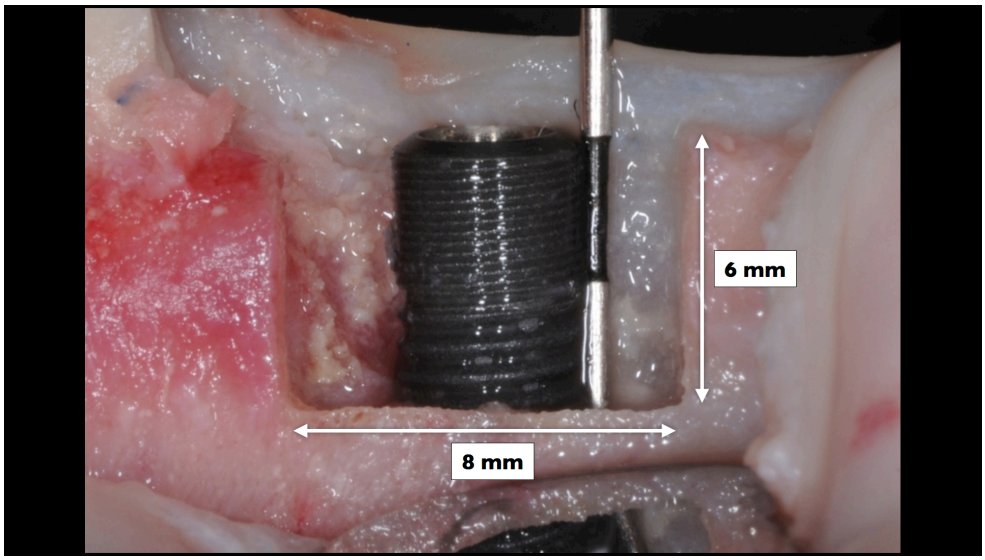
Figure 1

a)



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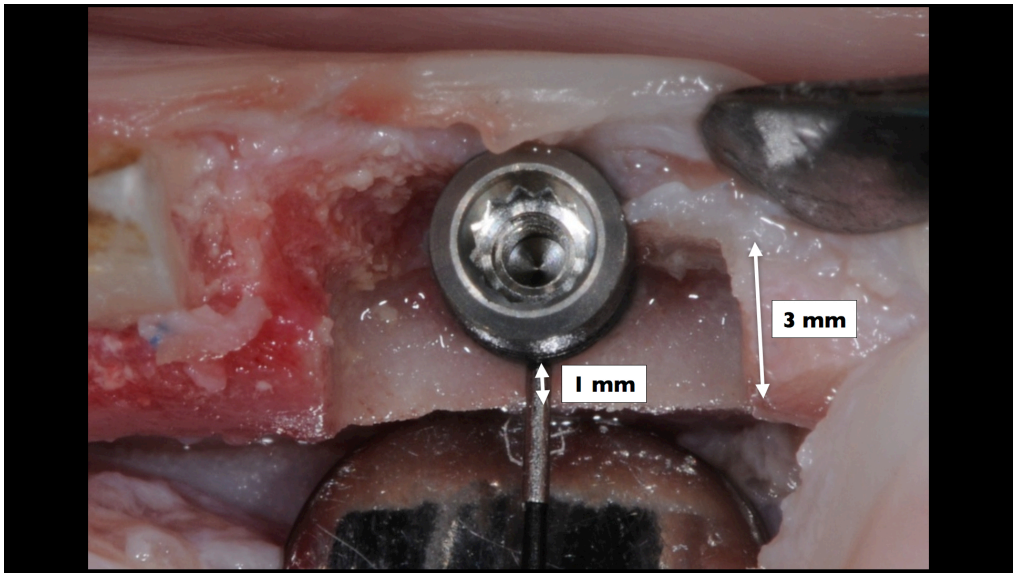
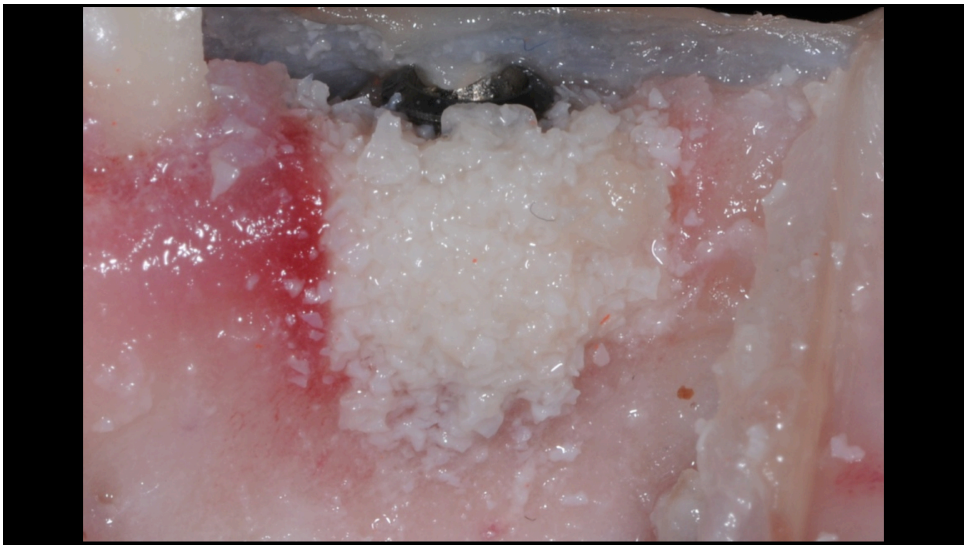


Figure 2

a)



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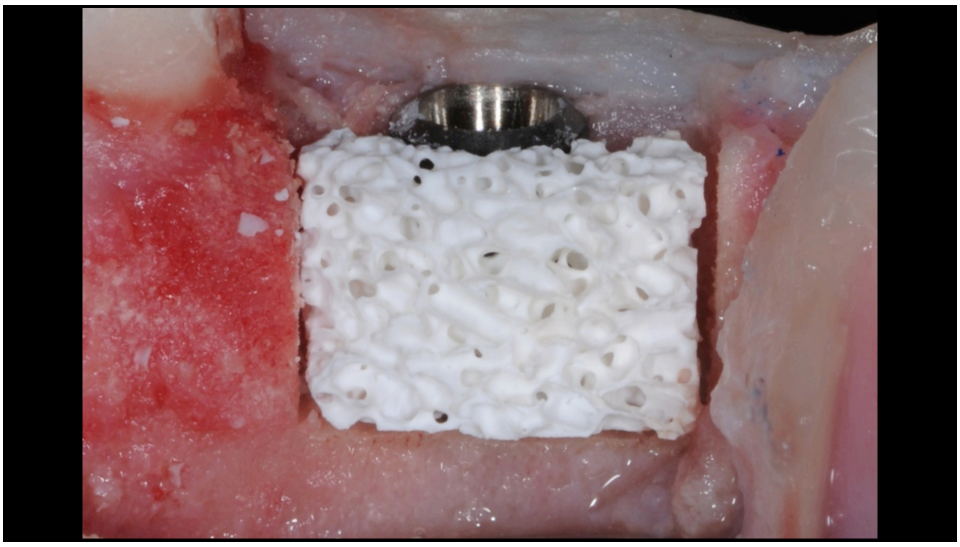

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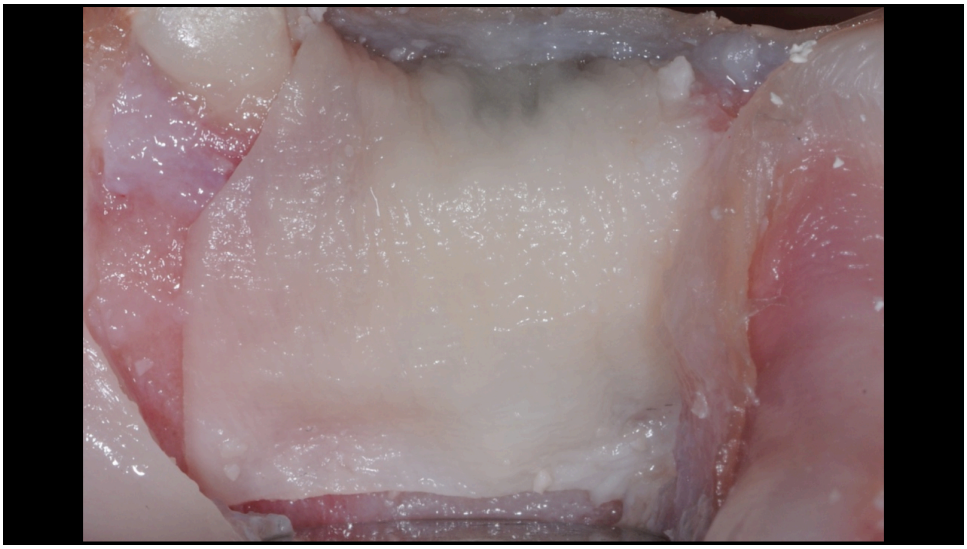

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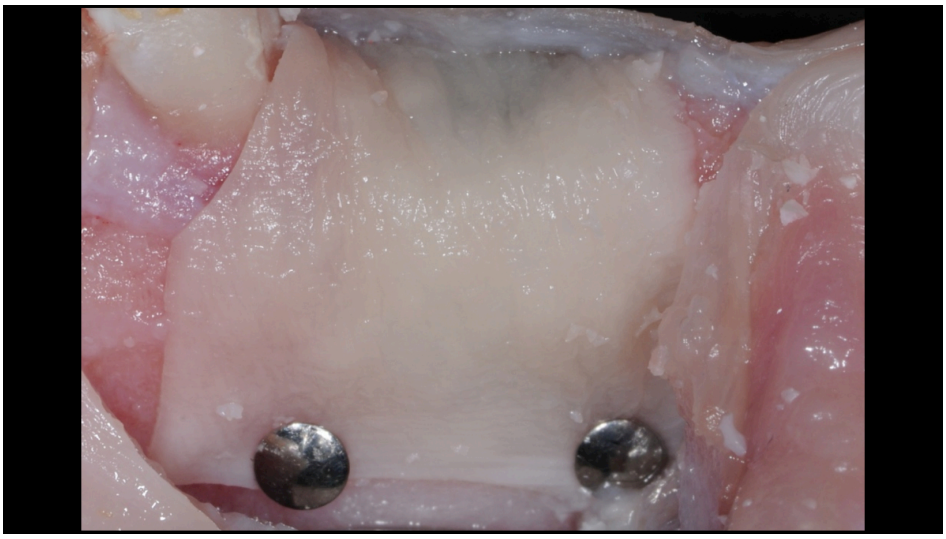

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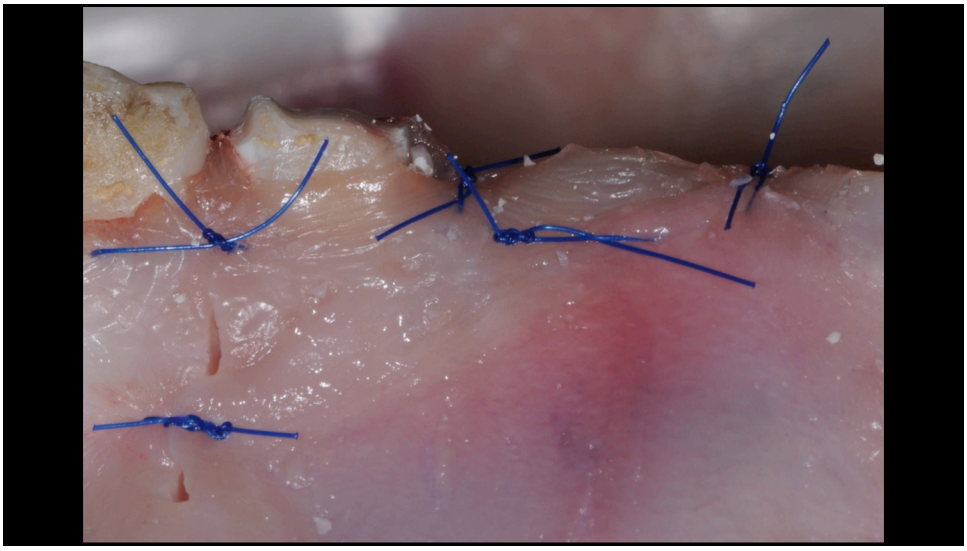
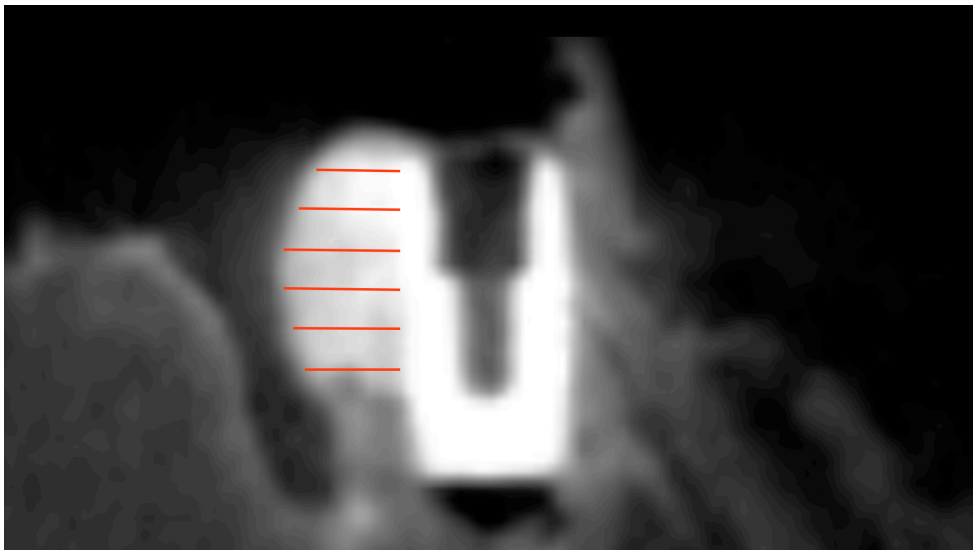


Figure 3

a)



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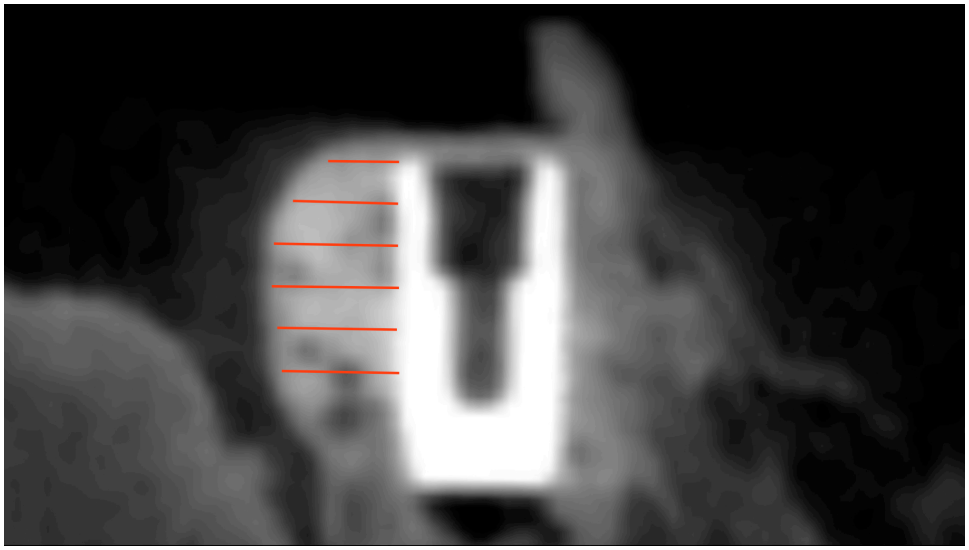

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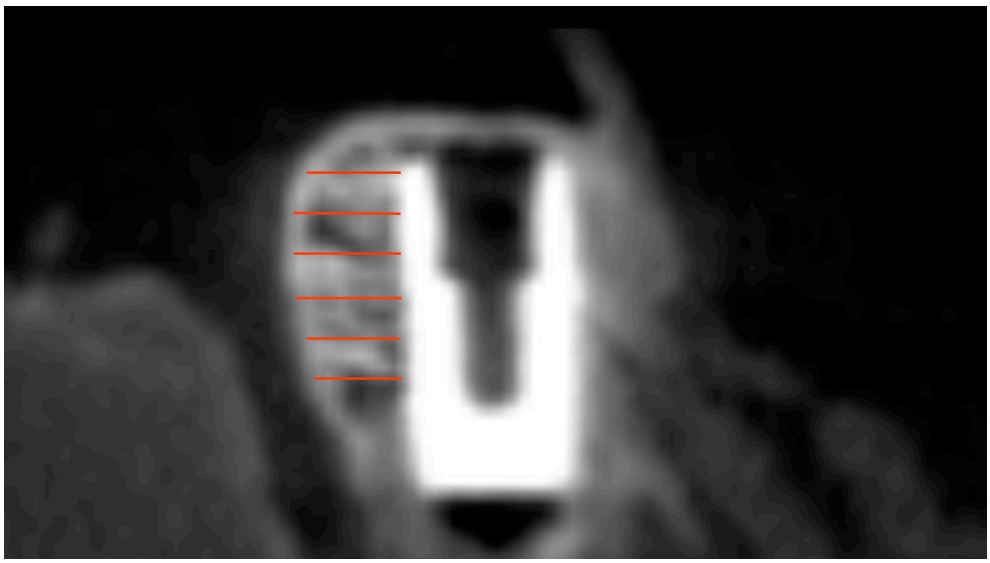

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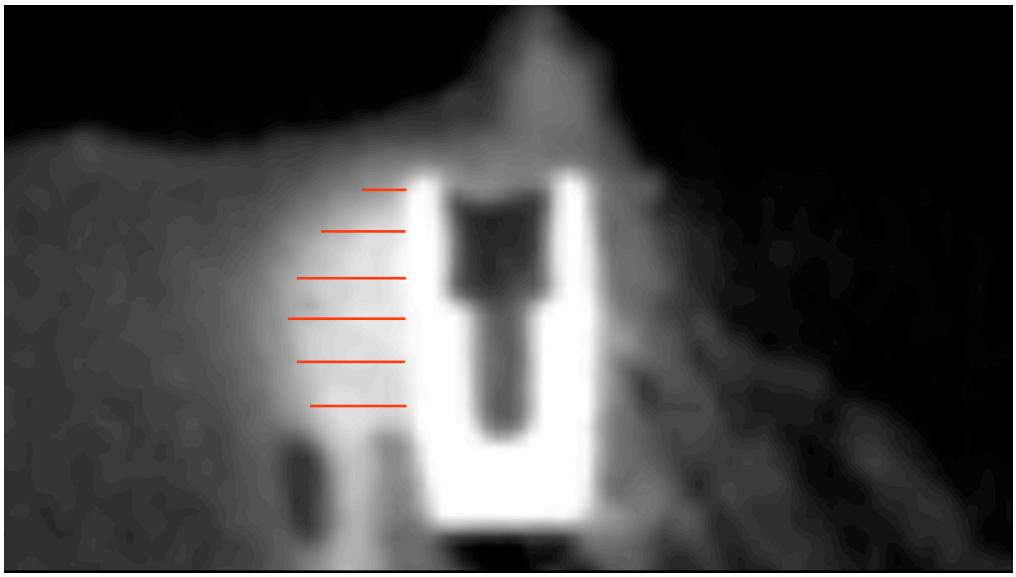

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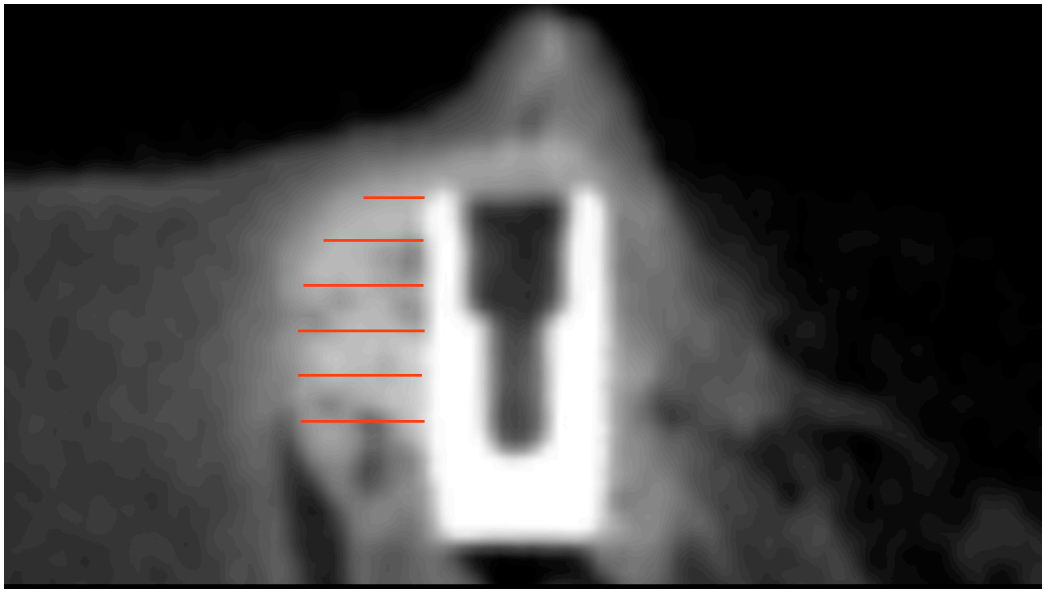

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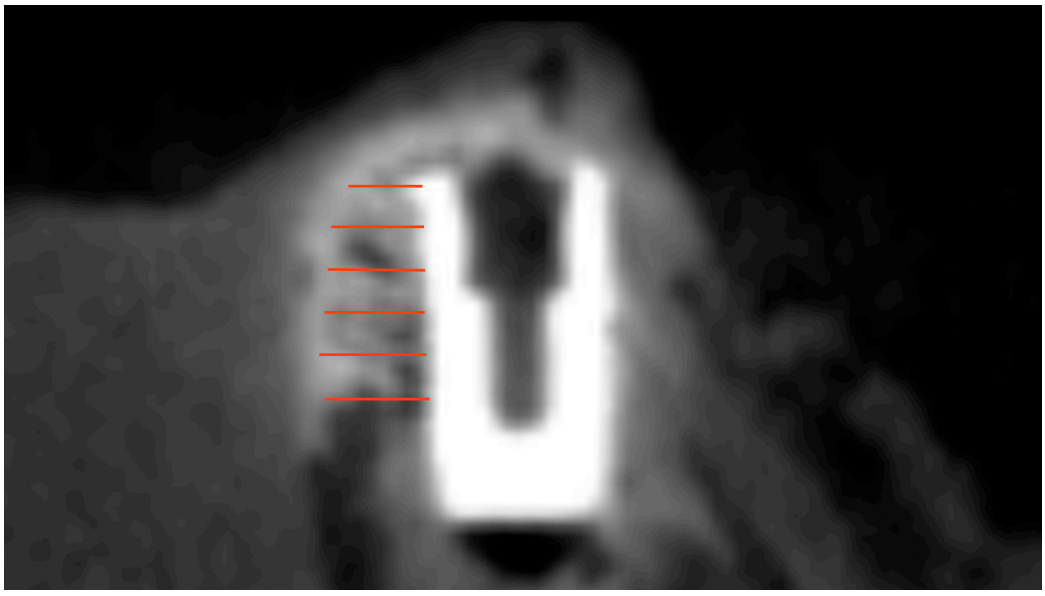
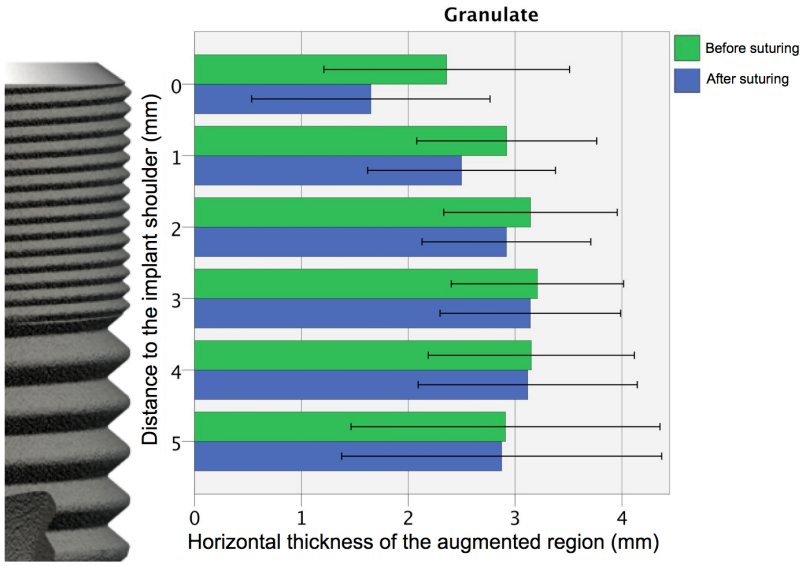
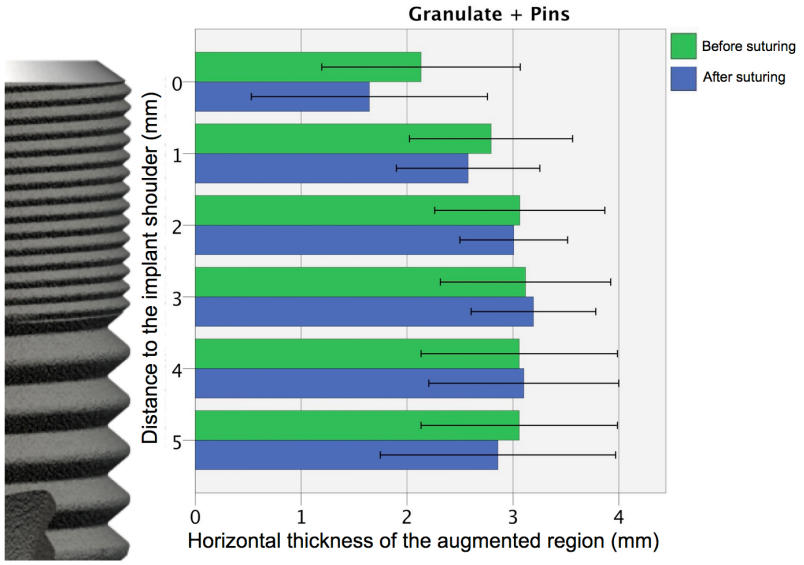


Figure 4

a)


Granulate
Before suturing
After suturing
Distance to the implant shoulder (mm)
0
1
2
3
4
5
0
1
2
3
4
Horizontal thickness of the augmented region (mm)

b)


Granulate + Pins
Before suturing
After suturing
Distance to the implant shoulder (mm)
0
1
2
3
4
5
0
1
2
3
4
Horizontal thickness of the augmented region (mm)

c)

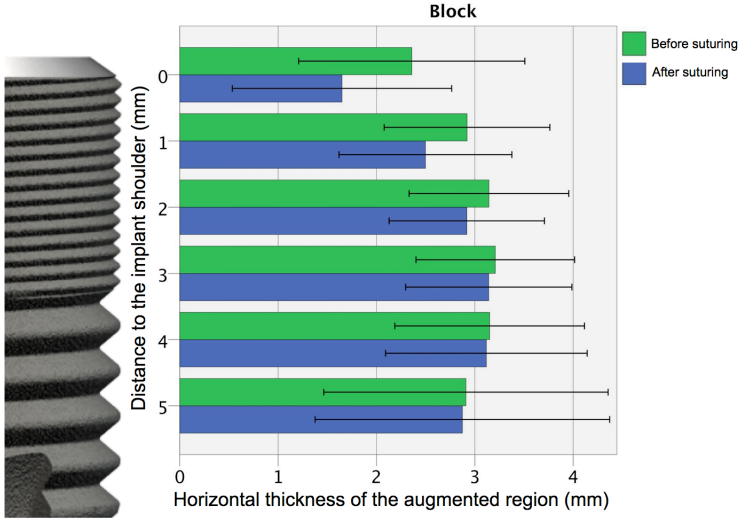
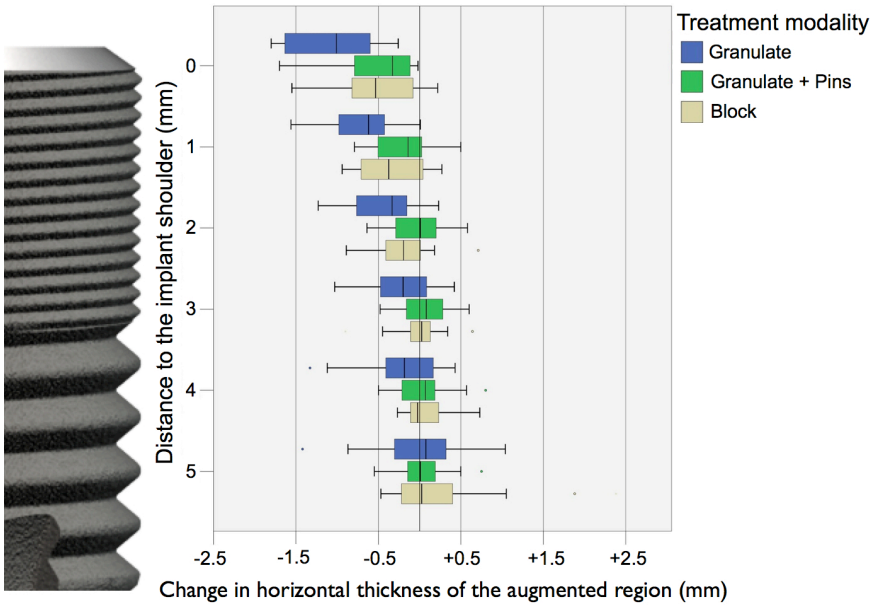

Block
Before suturing
After suturing
Distance to the implant shoulder (mm)
0
1
2
3
4
5
0
1
2
3
4
Horizontal thickness of the augmented region (mm)

Figure 5

a)


Treatment modality
Granulate
Granulate + Pins
Block
Distance to the implant shoulder (mm)
0
1
2
3
4
5
-2.5
-1.5
-0.5
+0.5
+1.5
+2.5
Change in horizontal thickness of the augmented region (mm)

b)

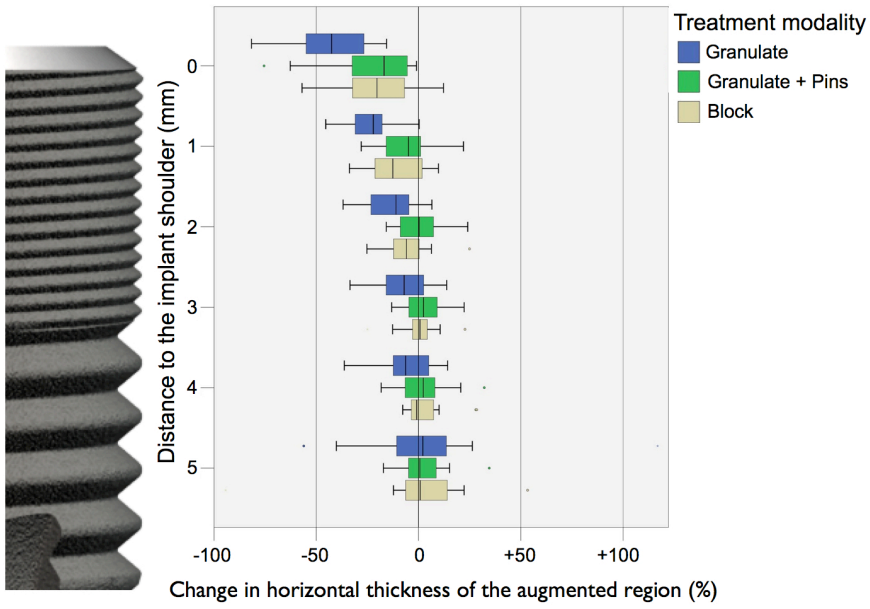

Treatment modality
Granulate
Granulate + Pins
Block
Distance to the implant shoulder (mm)
0
1
2
3
4
5
-100
-50
0
+50
+100
Change in horizontal thickness of the augmented region (%)

Table 1

Factor(s)	Mauchly's sphericity	F	Fd	p-value*
(1) Treatment modality§	0.829	1.153	2	0.328
(2) Apico-coronal level‡	< 0.001	41.108	1.749	< 0.001†
(1) and (2)	< 0.001	1.335	2.390	0.276

§, GBR procedures; ‡, 0-5 mm apical to the implant shoulder; F, Fischer's distribution; Fd, degrees of freedom; *, results of repeated measures ANOVA with Greenhouse-Geisser correction; †, statistically significant

Factor(s)	Mauchly's sphericity	F	Fd	p-value*
(1) Treatment modality§	0.674	1.059	2	0.358
(2) Apico-coronal level‡	< 0.001	68.531	1.397	< 0.001†
(3) Suturing	1	16.124	1	0.001†
(1) and (2)	< 0.001	1.826	3	< 0.001†
(1) and (3)	0.764	10.398	2	< 0.001†
(2) and (3)	0.003	24.780	2.219	< 0.001†
(1), (2) and (3)	< 0.001	1.755	2.794	0.172

§, GBR procedures; ‡, 0-5 mm apical to the implant shoulder; F, Fischer's distribution; Fd, degrees of freedom; *, results of repeated measures ANOVA with Greenhouse-Geisser correction; †, statistically significant

Table 2

Treatment modality	Parameter	Before suturing (mm)	After suturing (mm)	Change (mm)	Change (%)	p-value*
		Mean ± SD [95% CI]				
(a) Granulate (n=20)						
	HT_{0mm}	2.5 ± 0.6 [2.2; 2.8]	1.4 ± 0.5 [1.1; 1.6]	-1.1 ± 0.5 [-1.3; -0.8]	-42.8 ± 17.9 [-51.2; -34.4]	< 0.001†
	HT_{1mm}	3.0 ± 0.5 [2.8; 3.3]	2.3 ± 0.5 [2.1; 2.6]	-0.7 ± 0.4 [-0.9; -0.5]	-23.4 ± 11.9 [-28.9; -17.8]	< 0.001†
	HT_{2mm}	3.2 ± 0.5 [3.0; 3.5]	2.8 ± 0.5 [2.6; 3.1]	-0.4 ± 0.4 [-0.6; -0.2]	-13.1 ± 12.1 [-18.8; -7.5]	< 0.001†
	HT_{3mm}	3.3 ± 0.5 [3.1; 3.5]	3.0 ± 0.6 [2.8; 3.3]	-0.3 ± 0.4 [-0.5; -0.1]	-7.8 ± 12.4 [-13.6; -1.9]	0.012†
	HT_{4mm}	3.2 ± 0.6 [2.9; 3.5]	3.0 ± 0.7 [2.7; 3.3]	-0.2 ± 0.5 [-0.4; 0.0]	-6.6 ± 14.3 [-13.2; 0.1]	0.066
	HT_{5mm}	2.8 ± 0.8 [2.4; 3.2]	2.8 ± 0.9 [2.4; 3.2]	-0.0 ± 0.6 [-0.3; 0.3]	-2.0 ± 34.3 [-14.0; 18.1]	0.881
(b) Granulate+Pins (n=20)						
	HT_{0mm}	2.1 ± 0.5 [1.9; 2.4]	1.6 ± 0.6 [1.4; 1.9]	-0.5 ± 0.5 [-0.7; -0.3]	-22.9 ± 21.2 [-32.8; -13.0]	< 0.001†
	HT_{1mm}	2.8 ± 0.4 [2.6; 3.0]	2.6 ± 0.3 [2.4; 2.7]	-0.2 ± 0.4 [-0.4; -0.1]	-6.9 ± 12.5 [-12.8; -1.0]	0.012†
	HT_{2mm}	3.1 ± 0.4 [2.9; 3.3]	3.0 ± 0.3 [2.9; 3.1]	-0.1 ± 0.3 [-0.2; 0.1]	-0.9 ± 10.5 [-5.8; 4.0]	0.440
	HT_{3mm}	3.1 ± 0.4 [2.9; 3.3]	3.2 ± 0.3 [3.1; 3.3]	0.1 ± 0.3 [-0.1; 0.2]	3.3 ± 11.0 [-1.8; 8.5]	0.316
	HT_{4mm}	3.1 ± 0.5 [2.8; 3.3]	3.1 ± 0.5 [2.9; 3.3]	0.0 ± 0.4 [-0.1; 0.2]	2.2 ± 12.0 [-3.5; 7.8]	0.586
	HT_{5mm}	2.8 ± 0.6 [2.6; 3.1]	2.9 ± 0.6 [2.6; 3.1]	0.0 ± 0.3 [-0.1; 0.2]	1.6 ± 12.1 [-4.1; 7.3]	0.817
(c) Block (n=18)♠						
	HT_{0mm}	2.5 ± 0.6 [2.2; 2.8]	1.9 ± 0.5 [1.7; 2.2]	-0.6 ± 0.5 [-0.8; -0.3]	-20.2 ± 18.9 [-29.6; -10.8]	< 0.001†
	HT_{1mm}	2.9 ± 0.4 [2.8; 3.1]	2.6 ± 0.4 [2.4; 2.8]	-0.3 ± 0.4 [-0.5; -0.1]	-10.8 ± 13.2 [-17.4; -4.3]	0.002†
	HT_{2mm}	3.1 ± 0.3 [3.0; 3.3]	3.0 ± 0.4 [2.8; 3.1]	-0.2 ± 0.4 [-0.4; 0.0]	-5.3 ± 11.6 [-11.0; 0.5]	0.050†
	HT_{3mm}	3.2 ± 0.3 [3.0; 3.4]	3.2 ± 0.3 [3.0; 3.4]	-0.0 ± 0.3 [-0.2; 0.2]	-0.0 ± 9.8 [-4.8; 4.9]	0.856
	HT_{4mm}	3.2 ± 0.4 [3.0; 3.4]	3.3 ± 0.3 [3.2; 3.4]	0.1 ± 0.3 [-0.1; 0.2]	3.1 ± 10.6 [-2.2; 8.3]	0.330
	HT_{5mm}	2.9 ± 0.9 [2.4; 3.3]	3.0 ± 0.8 [2.6; 3.4]	0.1 ± 1.1 [-0.4; 0.7]	27.2♦ ± 89.5 [-17.3; 71.7]	0.435♣

n, number; HT_{xmm}, horizontal thickness of the augmented region measured x mm apical to the implant shoulder; ♠, two samples were excluded for Block procedure due to unclear contour of the augmented region; SD, standard deviation; 95% CI, 95% confidence interval; *, results of repeated measures ANOVA with Greenhouse-Geisser correction; †, statistically significant; ♦, the relative value of 27.2 % is driven by an outlier. If the outlier is excluded, the mean relative value of change in HT_{5mm} for Block amounts to 11.0 % ; ♣, results of non-parametric Wilcoxon test

Table 3

	Change (mm)			Change (%)			
	Granulate (G)	Granulate+Pins (P Block (B)		Granulate (G)	Granulate+Pins (P) Block (B)		Statistical analysis*
	Mean ± SD [95% CI]						
HT_{0mm}	-1.1 ± 0.5 [-1.3; -0.8]	-0.5 ± 0.4 [-0.7; -0.3]	-0.6 ± 0.5 [-0.8; -0.3]	-42.8 ± 17.9 [-51.2; -34.4]	-22.9 ± 21.2 [-32.8; -13.0]	-20.2 ± 18.9 [-29.6; -10.8]	G vs P: p < 0.001† G vs B: p = 0.011† P vs B: p = 0.498
HT_{1mm}	-0.7 ± 0.4 [-0.9; -0.5]	-0.2 ± 0.4 [-0.4; -0.1]	-0.3 ± 0.4 [-0.5; -0.1]	-23.4 ± 11.9 [-28.9; -17.8]	-6.9 ± 12.5 [-12.8; -1.0]	-10.8 ± 13.2 [-17.4; -4.3]	G vs P: p < 0.001† G vs B: p = 0.004† P vs B: p = 0.210
HT_{2mm}	-0.4 ± 0.4 [-0.6; -0.2]	-0.1 ± 0.3 [-0.2; 0.1]	-0.2 ± 0.4 [-0.4; 0.0]	-13.1 ± 12.1 [-18.8; -7.5]	-0.9 ± 10.5 [-5.8; 4.0]	-5.3 ± 11.6 [-11.0; 0.5]	G vs P: p = 0.001† G vs B: p = 0.008† P vs B: p = 0.216
HT_{3mm}	-0.3 ± 0.4 [-0.5; -0.1]	0.1 ± 0.3 [-0.1; 0.2]	0.0 ± 0.3 [-0.2; 0.2]	-7.8 ± 12.4 [-13.6; -1.9]	3.3 ± 11.0 [-1.8; 8.5]	0.0 ± 9.8 [-4.8; 4.9]	G vs P: p = 0.007† G vs B: p = 0.013† P vs B: p = 0.362
HT_{4mm}	-0.2 ± 0.5 [-0.4; 0.0]	0.0 ± 0.4 [-0.1; 0.2]	0.1 ± 0.3 [-0.1; 0.2]	-6.6 ± 14.3 [-13.2; 0.1]	2.2 ± 12.0 [-3.5; 7.8]	3.1 ± 10.6 [-2.2; 8.3]	G vs P: p = 0.132 G vs B: p = 0.034† P vs B: p = 0.913
HT_{5mm}	0.0 ± 0.6 [-0.3; 0.3]	0.0 ± 0.3 [-0.1; 0.2]	0.1 ± 1.1 [-0.4; 0.7]	-2.0 ± 34.3 [-14.0; 18.1]	1.6 ± 12.1 [-4.1; 7.3]	27.2♦ ± 89.5 [-17.3; 71.7]	G vs P: p = 0.867 G vs B♣: p = 0.862 P vs B♣: p = 0.760

HT_{xmm}, horizontal thickness of the augmented region measured x mm apical to the implant shoulder; SD, standard deviation; 95% CI, 95% confidence interval; *, results of repeated measures ANOVA with Greenhouse-Geisser correction; †, statistically significant; ♦, the relative value of 27.2 % is driven by an outlier. If the outlier is excluded, the mean relative value of change in HT_{5mm} for Block amounts to 11.0 % ; ♣, results of non-parametric Wilcoxon test

Table 4

Apico-coronal level*	Granulate		Granulate+Pins		Block	
	Before suturing	After suturing	Before suturing	After suturing	Before suturing	After suturing
0mm	0 % (0/20)	0 % (0/20)	0 % (0/20)	0 % (0/20)	0 % (0/20)	0 % (0/20)
1mm	5 % (1/20)	0 % (0/20)	0 % (0/20)	0 % (0/20)	0 % (0/20)	0 % (0/20)
2mm	0 % (0/20)	0 % (0/20)	5 % (1/20)	0 % (0/20)	0 % (0/20)	5 % (1/20)
3mm	10 % (2/20)	0 % (0/20)	0 % (0/20)	0 % (0/20)	20 % (4/20)	35 % (7/20)
4mm	5 % (1/20)	0 % (0/20)	5 % (1/20)	0 % (0/20)	15 % (3/20)	25% (5/20)
5mm	0 % (0/20)	0 % (0/20)	0 % (0/20)	0 % (0/20)	0 % (0/20)	0 % (0/20)

*, distance to the implant shoulder measured parallel to implant axis in the apical direction